An Aggressive Bone Marrow Evaluation Including Immunocytology With GD2 for Advanced Retinoblastoma

Guillermo L. Chantada, MD, Jorge Rossi, PhD,† Fernando Casco, MD,‡ Adriana Fandiño, MD,§ Marcelo Scopinaro, MD,* María T. G. de Dávila, MD,‡ and David H. Abramson, MD‖*

Summary: There is general agreement that bone marrow (BM) examination for staging in patients with retinoblastoma should be limited to cases with advanced disease. However, there are limited data about the yield of sampling multiple sites with aspirations and biopsies and immunocytology. Our policy for BM examination included: 2 aspirates and 2 biopsies at the posterior iliac crest scheduled only for cases with postlaminar optic nerve extension (n = 56), scleral invasion (n = 10) or orbital (n = 5) or metastatic disease at diagnosis (n = 7) or at extraocular relapse (n = 18). Immunocytology with the antibodies 3A7 or 3F8 for the ganglioside GD2 was performed. From 1/1994 to 3/2005, 277 newly diagnosed patients and 5 at extraocular relapse were included. BM invasion was not found in any of the 66 patients enucleated with disease confined to the globe, but was found in 11/27 of those with overt extraocular disease. There were 2/11 cases with at least 1 negative aspirate with positive biopsy and/or immunocytology for GD2. GD2 positivity was found in 9/9 cases. A more aggressive BM evaluation has a low yield in enucleated patients with high-risk features but disease limited to the globe. However, in cases with overt extraocular dissemination, the use of BM biopsy and immunocytology for GD2 allowed for the detection of cases that would have been missed by aspirations alone. GD2 was intensively expressed and it may also be used to monitor disease response and the presence of minimal residual disease.

Key Words: retinoblastoma, bone marrow biopsy, GD2, immunocytology, metastasis

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Bone marrow (BM) metastases are a hallmark of pediatric neuroectodermic tumors such as neuroblastoma, Ewing sarcoma, and retinoblastoma.[1–3] BM invasion occurs in up to 50% of patients with neuroblastoma, but it is less frequent in retinoblastoma.[1,2–7] However, BM metastases are a relatively common feature of disseminated retinoblastoma as it is seen in the developing countries. Disseminated retinoblastoma is uncommon in developed countries so, most reported series include few cases with BM involvement. Unlike other neuroectodermal tumors, there is little published information about the number of sites needed and the use of immunocytology for an accurate evaluation of the BM in retinoblastoma. Most previously reported studies of BM assessment in retinoblastoma refer to the use of single aspirates.[5–7] Recently, the experience of the Memorial Sloan Kettering Cancer Center in the evaluation of BM through 4 aspirations and 2 biopsies was published.[4] However, the yield of this aggressive screening was low, but only a few patients with extraocular disease were included to make definitive recommendations. A similar guideline for BM evaluation is performed at the Hospital JP Garrahan (Argentina) where more cases with advanced disease are seen, so we decided to evaluate and compare our experience.

Immunocytologic evaluation of the BM aspirates may further improve the sensitivity of BM cytology and histology.[8] However, there are limited published data on retinoblastoma. The immunocytologic detection of the ganglioside GD2 in the BM of patients with neuroblastoma has been published, but little information exists for retinoblastoma.[8–10] From 1995 onwards, the 3A7 and later the 3F8 antibodies facilitated by the Memorial Sloan Kettering Cancer Center for immunocytologic detection of GD2 positive cells was also included in the BM evaluation at our center.

Therefore, the aim of this study is to describe the results of this guideline for BM examination in patients with retinoblastoma and the expression of GD2 in a cohort including patients with advanced stage with higher risk for BM involvement.

PATIENTS AND METHODS

All newly diagnosed patients with retinoblastoma from January 1994 to March 2005 were included. All cases were included into prospective treatment protocols. Because the Hospital JP Garrahan is a tertiary care institution, we have also received patients for the treatment of extraocular relapse and were not eligible for these protocols, but included for this report. BM

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From the Departments of *Hemato-oncology; †Immunology; ‡Pathology; §Ophthalmology, Hospital JP Garrahan, Buenos Aires, Argentina; and ‖Department of Ophthalmic Oncology, Memorial Sloan Kettering Cancer Center. New York, NY.
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Reprints: Guillermo L Chantada, MD, Hemato-oncology Department, Hospital JP Garrahan, Combate de los Pozos 1881, C1245AAL, Buenos Aires, Argentina (e-mail: gchantada@garrahan.gov.ar).
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TABLE 1. The Grabowski-Abramson Classification[11]

I. Intraocular disease
  (a) Retinal tumors
  (b) Extension into to the lamina cribrosa
  (c) Uveal extension
II. Orbital disease
  (a) Orbital tumor
    (1) Scattered episcleral cells
    (2) Orbital invasion
  (b) Optic nerve
    (1) Tumor beyond the lamina but not up to cut section
    (2) Tumor at the cut section of optic nerve
III. Intracranial metastasis
  (a) Positive cerebrospinal fluid only
  (b) Mass CNS lesion
IV. Hematogenous metastasis
  (a) Positive marrow-bone lesions
  (b) Focal lesions with or without positive marrow
  (c) Other organ involvement

evaluation was restricted to patients with high-risk features. Thus, it was carried out only in those cases with Grabowski-Abramson (Table 1)[11] stage II (orbital invasion or postlaminar optic nerve) or greater and those with an overt extraocular relapse. All patients were scheduled to undergo evaluation at four BM sites [2 biopsy specimens (right and left posterior iliac crest) and 2 aspirates from different sites] using different needles for each site, shortly after diagnosis and before initiation of systemic treatment. The procedures were carried out under anesthesia or conscious sedation in all patients. No follow-up BM examination was scheduled in patients with negative findings. Approximately, 10 marrow smears were performed and stained with standard May-Grunwald-Giemsa stain and at least 4 of them were assessed by an experienced pediatric oncologist. About 1 to 2 mL of marrow was collected at each site in the same heparinized syringe and examined by immunofluorescence for GD2 expression using the 3A7 or 3F8 antibodies as previously reported.[8] These antibodies were provided by Dr NK Cheung (Memorial Sloan Kettering Cancer Center, NY) from 1995 onwards. In patients with extensive extraocular disease in poor clinical condition, a single aspirate with GD2 determination was allowed and if negative for tumor, a complete evaluation as described above was scheduled if patient's clinical condition was stable enough to safely tolerate general anesthesia. One patient of this series underwent a BM examination, because of abnormal counts and hepatosplenomegaly and final diagnosis was secondary acute nonlymphoblastic leukemia.[12] All pathology slides were retrieved and reanalyzed for this study by 2 pathologists (F.C. and M.T.G.D.) in a masked fashion.

All patients in whom BM was investigated also had a brain CT scan and lumbar puncture with examination of the cytospin performed. Patients with clinical evidence of bone metastasis underwent a bone scintigraphy. Abdominal ultrasound and chest x-rays were performed in most patients. Relevant clinical data were retrieved from our database and complemented with chart review.

RESULTS

A total of 277 patients with newly diagnosed retinoblastoma were seen at our hospital during the study period. There were 5 additional patients referred from other institutions for the treatment of extraocular relapse and were analyzed for this report. Eleven of the 282 patients had BM invasion at diagnosis[3] or extraocular relapse[8] (Table 2). Seven of them had unilateral retinoblastoma. There were 5 male and 6 female.

BM Examination in Enucleated Patients With Pathologic Risk Factors

BM examination was performed for staging purposes in all 66 enucleated patients with high-risk features but disease limited to the globe at diagnosis (Table 2). BM invasion was not detected in any case.

BM Examination in Patients With Overt Extraocular Retinoblastoma

There were 12/277 patients with overt extraocular retinoblastoma at diagnosis (Grabowski-Abramson stage showed in Table 2). Three of them had BM invasion. Eighteen patients with overt extraocular relapse were included. BM examination was not performed in 3 of them with extensive relapse involving the central nervous system (CNS) and they were treated palliatively. Five patients in this group were initially treated at other institutions and referred to our center for the treatment of relapse and all had BM invasion upon examination at our center. The remaining 13 were treated at our institution

TABLE 2. BM Evaluations According to Stage

Grabowski-Abramson Stage	N	Positive Aspirations	Positive Biopsies	Positive Immunocytology
IIa1 (scattered episcleral cells)	8	None	None	None
IIa2 (microscopical orbital tumor)	2	None	None	None
IIb1 (Tumor beyond the lamina but not up to cut section)	49	None	None	None
IIb2 (Tumor at the cut section of optic nerve)	7	None	None	None
IIb2 (Tumor at the cut section of optic nerve AND overt orbital dissemination)	5	None	None	None
III (Intracranial metastasis)	3	None	None	None
IV (Hematogenous metastasis)	4	3	3	2
Extraocular relapse*	18	8/15	6/13	7/13

*BM examination was not carried out in 3 cases.

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and BM invasion was detected in 3 patients. No information about eye pathology or BM findings at diagnosis could be retrieved for the 5 patients treated elsewhere. Of the 3 relapsed patients with BM invasion treated initially at our institution, BM was negative at diagnosis in 2 with postlaminar optic nerve invasion (stage IIb1). The remaining case had intraocular disease at diagnosis so BM was not assessed at that time. Therefore, 3/12 patients with overt extraocular disease at diagnosis and 8/15 cases of extraocular relapse in whom BM was investigated had BM metastases.

BM Aspirations, Biopsies, and Immunocytology With GD2 (Table 3)

A single BM aspirate was carried out in 2 patients with overt metastatic relapse and it was positive for tumor cells in both. There were 2 patients in whom there was at least 1 aspirate negative for tumor together with a positive biopsy and immunocytology. In 1 of them (no. 5), a few suspicious clumps were evident but they were not conclusive for tumor. Both immunocytology for GD2 and biopsy were positive. In the remaining one (no. 11) with multiple bone metastases, there was 1 aspirate and biopsy negative from the right side that were negative for tumor and both aspirate and biopsy were positive in the left side. This case was seen before the use of immunocytology.

Immunocytology for GD2 was not carried out in 2 patients but it was positive en 9/9 cases with BM involvement. GD2 positivity was always intense. GD2 was never positive with normal BM findings.

Treatment

There were 2 patients with BM invasion that were treated palliatively and both died within 1 month of diagnosis of BM invasion. Seven patients were treated with a curative intent with conventional chemotherapy before high dose chemotherapy and autologous stem cell rescue became widely available. The response to treatment was evaluated at the BM in 4 and all achieved a complete clearance of tumor cells after chemotherapy. However, all died of relapse or progressive disease usually in the CNS. The latest 2 patients received consolidation with high dose chemotherapy and autologous stem cell rescue after achieving a complete response to conventional chemotherapy. In both a complete BM examination showed negativity for tumor cells and GD2 before transplantation. Both survive disease-free (+8 and 28 mo).

DISCUSSION

Our results confirmed the Memorial Sloan Kettering experience that a more aggressive BM screening in retinoblastoma did not increase the yield in a larger cohort of enucleated patients with retinoblastoma and high-risk features but with disease limited to the globe.[4] However, in patients with overt extraocular disease, a more extensive work up including 2 aspirations and 2 biopsies together with immunocytology for GD2 allowed

TABLE 3. Description of 11 Patients With BM Invasion Included in our Study

Patient (Laterality)	Extraocular Sites	Treatment	Prior Therapy	Biopsy	Aspiration	GD2	CBC	Status
1 (B)	Orbit, CNS, liver, pancreas	No	Chemoreduction EBRT. Not enucleated	N/D	Massive invasion (single aspirate)	+	Peripheral blasts, thrombocytopenia, erithroblastosis	DOD
2 (B)	Bone, CNS	Chemotherapy	None	Both +	Massive invasion	N/D	Normal	DOD
3 (U)	Bone	Chemotherapy and ASCR	Enucleation	Both +	Massive invasion	+	Normal	ADF (+32)
4 (U)	Orbit, liver	Palliative	Enucleation, chemotherapy	N/D	Massive invasion (single aspirate)	+	Normal	DOD
5 (U)	Orbit	Chemotherapy and ASCR	Enucleation	Both +	Suspicious single cells	+	Normal	ADF (+8)
6 (B)	Orbit, lymph nodes	Chemotherapy	Chemoreduction, EBRT, enucleation	Both +	Massive invasion	+	Thrombocytopenia, erythroblastosis	DOD
7 (U)	Orbit	Chemotherapy	Enucleation	Both+	Rossettes	+	Normal	DOD
8 (B)	Orbit	Chemotherapy	Chemoreduction	Both +	Massive invasion	+	Leukocytosis	DOD
9 (U)	CNS, bone, orbit	Chemotherapy	None	Both+	Massive invasion	+	Normal	DOD
10 (U)	Orbit, CNS	Chemotherapy	None	Both+	Rossettes	+	Normal	DOD
11 (U)	Bone	Chemotherapy	Enucleation, chemotherapy	Left side +	Left side +Massive invasion	N/D	Normal	DOD

Patient nos. 2, 9, and 10 had BM invasion at diagnosis and the remaining ones at relapse. Patient nos.4, 6, 7, 8, 10, and 11 were treated initially at other institutions.
*Only 1 aspirate was positive for malignant cells.
ADF indicates alive disease-free; ASCR, autologous stem cell rescue; B, bilateral retinoblastoma; CBC, complete blood cell count; DOD, died of disease; EBRT, external beam radiation therapy; N/D, not done; U, Unilateral retinoblastoma.

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for the detection of cases of BM involvement that would have been missed with single aspirates.

Pratt et al[5] first reported that BM examination is not necessary for all patients with retinoblastoma for staging purposes. In their study, a single BM aspirate was carried out in 115 patients with retinoblastoma, but only 3 of 17 patients with regional extraocular or disseminated disease had BM metastasis. Despite BM metastasis only occurred in this subgroup, the authors recommended that BM examination should be performed for staging in other high-risk patients including those with choroidal, ora serrata, ciliary body, or the anterior chamber invasion and those with involvement other extraocular structures including the orbit, the optic nerve or when symptoms or when imaging studies suggest invasion of bones, soft tissue, or the CNS.[5] The infrequency of BM invasion in patients without the evidence of extraocular disease was subsequently confirmed by other authors in larger cohorts[6,7]; so this policy for BM evaluation became the standard of care. However, most studies reported on the use of a single BM aspirate as the recommended staging procedure. Like neuroblastoma, BM in retinoblastoma may be spotty,[2,11] so evaluation of multiple sites may increase the yield of detection of marrow metastasis. In 1987, Grabowski and Abramson[11] from New York suggested that BM aspiration and biopsies from more than 1 site would be necessary. The experience of the same group at Memorial Sloan Kettering Cancer Center with a more extensive BM work up was recently published.[4] Because only 11 patients with high-risk features were included in that series, we decided to investigate the results of a similar, extensive BM examination policy in Argentina, where more patients with high-risk features are seen. Both our institutions follow a similar philosophy of many aspects of care in patients with retinoblastoma and other collaborative studies were performed during that period.[12–14] Although the Memorial Sloan Kettering Cancer Center performed 4 aspirations and 2 biopsies for every patient with retinoblastoma, only patients with Grabowski-Abramson's stage II onwards and all patients with extraocular relapse underwent BM aspiration and biopsies from 2 different sites at the Hospital Garrahan. Immunocytologic evaluation with the 3A7 or 3F8 antibodies developed at Memorial Sloan Kettering Cancer Center for the detection of GD2 positive cells was also included in the BM evaluation at the Hospital Garrahan. However, despite our more aggressive BM examination policy and the inclusion of 66 patients with high-risk features, BM invasion was not detected at diagnosis in any case when disease was limited to the globe. Most children with high-risk features received adjuvant chemotherapy, so their natural history cannot be ascertained. Two patients in this category had an extraocular relapse and the BM was involved in both despite being negative at diagnosis. They had not received adjuvant therapy after enucleation.

In our study, an extensive BM evaluation including multiple sites and immunocytology increased the yield only for patients with overt extraocular retinoblastoma. In our series, a single BM aspirate might have been insensitive to rule out metastasis, because 2 cases had at least 1 negative aspirate. Most of our patients had normal peripheral blood counts, so peripheral blood findings are not reliable to predict which patient has BM involvement. Some of our patients with BM metastasis had concomitant orbital or lymph node involvement as their only extraocular site. Because these patients can be cured with conventional chemotherapy and orbital radiotherapy,[15] it is critical to effectively rule out BM invasion because a more intensive therapy including high dose chemotherapy and autologous stem cell rescue is needed for better results.[16] The determination of complete response in the BM is important in patients with BM invasion before undergoing autologous stem cell rescue to detect possible contamination with tumor cells. The use of immunocytology for GD2 and an additional number of sites proved to increase the sensitivity in neuroblastoma.[8] Only 2 cases were studied in our series, so this policy of BM evaluation may be insufficient for the evaluation of response before high dose chemotherapy and autologous stem cell rescue. Our results show that patients with retinoblastoma and BM invasion are seldom curable with conventional chemotherapy, but may become long-term survivors with high dose chemotherapy and autologous stem cell rescue.[16]

Our data also adds more evidence for the expression of GD2 in retinoblastoma. Portoukalian et al[17] reported that serum levels of GD2 were elevated in 9 out of 10 patients with retinoblastoma. GD2 was also detected in enucleated eyes with retinoblastoma and in the inner layer of the retina of unaffected animals (Lee TC, personal communication). GD2 detection by the use of the 3A7 antibody for diagnostic purposes in the BM of a patient with retinoblastoma was described previously in a single case report.[10] Our study confirmed these observations showing a high frequency of GD2 expression using specific antibodies such as the 3A7 and the 3F8 in a larger cohort. GD2 positivity was evident in all patients with BM involvement, including the case with negative aspirations and positive biopsy for tumor. Therefore, the use of these antibodies may increase the sensitivity of BM examination in patients with retinoblastoma. However, our relatively small sample size precluded the estimation of accurate sensitivity and specificity of the procedure. Detection of minimal residual disease using the GD2 synthase determination by real time polymerase chain reaction was described for neuroblastoma and according to our results showing high expression of GD2 in retinoblastoma it could be an interesting alternative for this tumor.[18]

To conclude: A more extensive BM examination with at least 2 aspirates and 2 biopsies from different sites may be indicated for extent of disease evaluation only for patients with overt extraocular retinoblastoma. Its yield for patients with disease limited to the globe even when pathologic risk factors were present is minimal. The use of immunocytologic detection of specific antigens like GD2 may also increase the yield of the procedure and help for evaluating response to the treatment.

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